MICTURITION IN THE CRAYFISH AND FURTHER OBSERVATIONS ON THE ANATOMY OF THE NEPHRON OF THIS ANIMAL

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Preliminary to studies on renal function in the crayfish (Maluf, 1940, 1941*b*), it is necessary to know how urine is retained in the bladders and how discharged. Nothing has been indicated, until the present, as to how urine is retained. There is, furthermore, no adequate study of the anatomical features surrounding the urinary outlet of decapod Crustacea. As a result of this deficit, investigators of renal function in the crayfish have punctured the membranous operculum at the nephropore prior to collecting urine by suction (Marchal, 1892; Boivin, 1929; Herrmann, 1931; Scholles, 1933; Lienemann, 1938). It is not clear why the opercula were destroyed. From Marchal's diagrams it appears that removal of the opercula would tear the ureters and lead into the haemocoele and that, consequently, the urine would be contaminated with blood. Marchal and Boivin, however, stated that the liquid they collected was limpid, clear, and almost colorless and practically uncontaminated with blood. The chemical analyses of Herrmann, Scholles, and Lienemann show that the concentration of inorganic electrolytes in the liquid collected from the excretory orifices was markedly lower than in the blood. The fact that the distal portion of the bladder contacts the base of the excretory eminences at most of its circumference (Fig. 2, *B*) apparently explains how the urine collected by the afore-mentioned investigators did not contain an appreciable quantity of blood. The urine aspirated by Picken (1936), by piercing the operculum with a fine hypodermic needle, was doubtless, at times at least, notably contaminated with blood as shown by the strongly positive xanthoproteic reaction and by the large discrepancies, in this respect, with regard to the urine from both kidneys. Thus, in one instance, the urine from the right kidney gave a negative xanthoproteic test while that from the left gave a strong reaction. The writer found that urine collected from *Cambarus clarkii* by suction from intact nephropores invariably gave a weak xanthoproteic but a negative biuret reaction.

The review of Burian and Muth (1924) may leave one with the impression that the communication between the coelomosac and labyrinth "is closed by a sphincter muscle, and any passage of fluid from the labyrinth into the coelomic sac appears to be prevented by a valve-like arrangement of cells" (Picken, 1936). Examination of the literature left the writer dubious about the existence of a sphincter between the coelomosac and labyrinth. The present paper shows that, at least in *Cambarus clarkii,* there is no sphincter or valve between coelomosac and labyrinth or between nephric tubule and bladder.

Weismann (1874), Grobben (1881), Schlieper (1935), and Peters (1935) believed that a blood-ultrafiltrate is formed in the coelomosac. The writer has made a detailed histological examination of this part of the nephron to find out whether the histological facts support the hypothesis of filtration.

The results in this paper refer to *Cambarus clarkii,* the swamp crayfish.

Micturition

The Retention of Urine

Because the bladders are normally distended with urine and because urine only occasionally leaves the nephropores of undisturbed unheated animals seen under a microscope, urine must be adequately retained in the bladders. The volume of retained urine was sometimes as much as 4 per cent of the fresh weight of the animal.

On the ventral surface of the basal segment of each second antenna is the whitish excretory eminence (Fig. 5, *e*) in the central depression of which is a convex, finely corrugated, flexible, thin membrane, *o,* known as the operculum. Because the operculum does not cover anything external, the name is inaccurate. The convexity of the operculum is maintained by blood-pressure, as the opercula invariably collapse after thoroughly bleeding the animal. In contrast to the rest of the excretory eminence, the operculum is very sensitive to contact as shown by the resulting generalised motor response. The operculum is invaginated at its anterior border, thus forming a narrow crescentic slit (Figs. 2, 3, 5, and 6, *a*) which is the actual excretory orifice, or nephropore. The invagination proceeds at a sharp angle posteriorly, forming the short flat ureter (Figs. 2 and 3, *ur*).

The rounded flexible contour of the operculum is inessential. An animal with both opercula damaged by puncture was under observation for about a month, at the end of which time its opercula were still collapsed. The ureters, however, were not damaged, as shown by subsequent dissection.

The ureter (Figs. 2 and 3, *ur*) is short, dorso-ventrally flattened, and parallel to the operculum. Fine spindle-shaped fibers (Figs. 2 and 3, *f'*) containing elongate nuclei (13 μ long in crayfish-saline) extend from the dorsal wall of the ureter to the basal margins of the excretory eminence. With care, the whole dorsal wall of the ureter, including the fibers, may be dissected and mounted.

The fibers are unstriated (observed at 970 $\times$ while in fresh saline or after being fixed in formalin and stained with haematoxylin or Wright's) and are apparently identical with those which stretch between the distal extremity of the bladder and the integument (Fig. 2, *f*, *f''*). These ureteral fibers apparently act as a sphincter and their discovery answers the question as to how urine is retained in the bladders. Because a gentle outflow of urine has been seen in deviscerated inverted animals, the bladder must be elastic and the ureteral sphincter evidently normally retains urine in the bladder by tonic contraction.

Similar fibers occur, circularly arranged in considerable numbers, on the haemocoelic surface of the most proximal portion of the bladder, to a very much slighter degree on the main body of the bladder, and also on the main stem of the renal artery. Spindle-shaped unstriated fibers have been observed on the bladder of the American lobster by Waite (1899).

PLATE I

FIG. 1. Dorsal aspect of the opening into the left second antenna and surrounding exoskeleton, showing the distal portion of the bladder wedged between the proximal antennal muscles. *am*, articular membrane between antenna and cephalothorax; *B*, distal portion of bladder; *bas*, basipodite; *c*, lateral wall of the cephalothorax; *comp*, compressor muscles of the antenna; *cox*, coxopodite; dep_{1-4}, depressor branches of the antenna; *lev*, levator muscle of the antenna; *prom*, promotor muscle of the antenna; *rem*, remotor muscle of the antenna; *s*, sternum.

FIG. 2. Sagittal section through the distal portion of the bladder, ureter, and nephropore. *B*, distal portion of the bladder; *c*, connective tissue; *f*, *f'*, *f''*, unstriated fibers; *f'*, ureteral sphincter; *n*, nephropore; *o*, operculum; *s*, coxopodite; *ur*, ureter.

FIG. 3. Dorsal aspect of the ureter and the depression of the coxopodite which corresponds to the eminence of the ventral aspect. *a*, nephropore, shown in broken lines because it is ventral to the ureter; *f'*, ureteral syncytium; *ur*, ureter.

FIG. 4. Dorsal aspect of the brain. *a*, nerve-stems passing into the lumen of the second antenna; c_1, c_2, individual nerve-fibers issuing from the roots of the former; *lc*, longitudinal connectives; *m*, median nerve; *oc*, oculomotor nerve; *op*, optic nerve; *P*, protocerebrum; *T*, tritocerebrum; *te*, "tegumentary" nerves. The root of the nerve to the first antenna issues from the ventral surface of the brain and is thus not shown here.

FIG. 5. Ventral aspect of the region surrounding the right nephropore. *a*, crescentic nephropore; *e*, excretory eminence of the basal segment of the second antenna; *o*, operculum; *s*, coxopodite; *u*, droplet of urine.

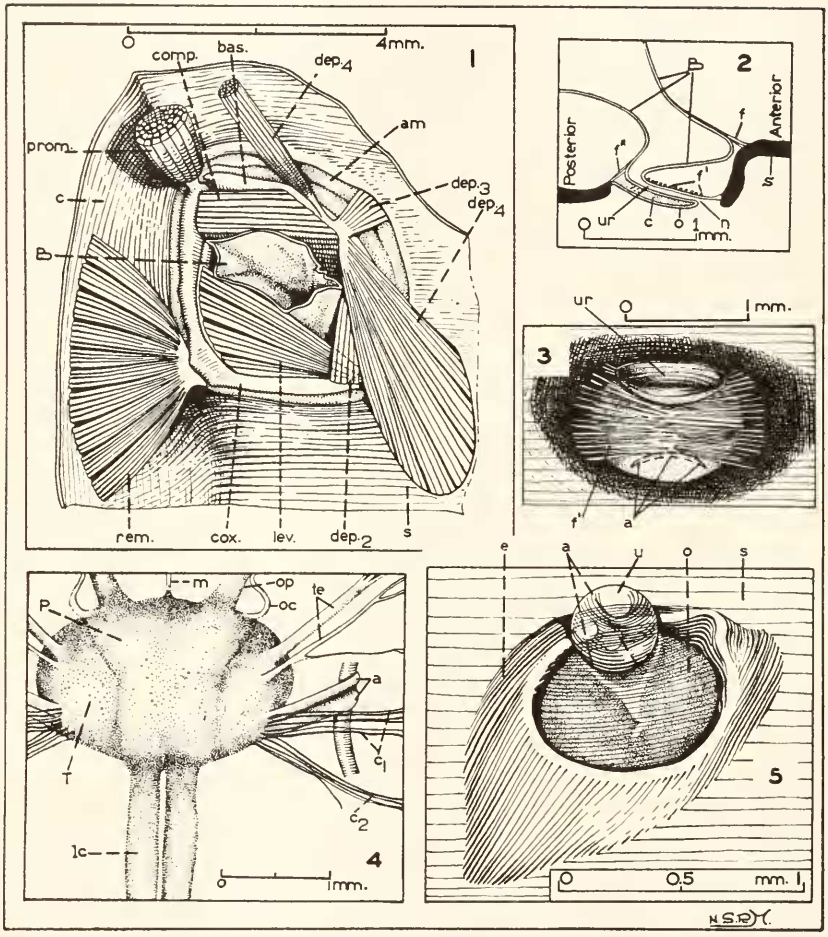

PLATE I

(All figures refer to *Cambarus clarkii;* the animals of Figs. 1, 4, and 5 measured about 7.5 cm. from rostrum to end of telson.)

In spite of numerous careful dissections, the writer has not been able to find any fibers inserting on the operculum. This conforms with Marchal's (1892) observations on the crab, *Maia.* Schmidt (1915), who gave a comprehensive and well-illustrated account of the somatic musculature of the European crayfish, did not mention any special muscles of micturition. The region between the operculum (Fig. 2, *o*) and the ureter, *ur,* is occupied by connective tissue and does not contain spindle-fibers.

At the posterior margin of the excretory eminence the ureter bends sharply anteriorly, enlarges in girth, and continues as the bladder (Fig. 2, *B*). Upon emerging from the excretory eminence (Fig. 2), the bladder passes through a mass of antennal muscles (Fig. 1).

The Expulsion of Urine

The animal was drained of moisture, water was sucked from the branchial chambers, and the anterior border of the chambers plugged with absorbent cotton-wool to prevent remaining water from flowing over the opercula. The opercula were observed under magnifications of 22.5 and 112.5 ×.

The outflow of urine in air occurs anteriad, i.e. in the plane of the ureter. At times the urine issues from the orifice for a short distance and is then sucked back. Slight pressure on the operculum with a blunt instrument frequently induces urinary outflow. A pipette of suitable size and carrying suction (about 10 mm. Hg) may produce urination even for some time after the use of suction. The urine issues as a series of generally spherical droplets. The suction does not injure the operculum. The latter does not undergo movement except for a scarcely perceptible motion only as the urinary droplet attains maximal size. This is doubtless a passive effect. Marchal (1892) stated that, in the crab *Maia,* movements of the opercula accompany the discharge of urine; it is probable that in *Maia,* too, the motion is passive. Marchal stated that muscles do not insert on the operculum of *Maia.* The writer confirms this for the crayfish.

Not infrequently, while the animal was held dorsum down and both nephropores were apparent, fine jets of urine abruptly spurted from both orifices sometimes to a distance of a foot or more. Every jet consisted of droplets in quick succession. On one occasion the occurrence was especially striking in that a series of jets to at least a foot followed one another rapidly. Although the spurts from both nephropores generally were not entirely simultaneous, the writer cannot recollect any instance in which urine spurted from one nephropore and not from the other

within a brief interval of time. Such powerful and sudden jets cannot be accounted for by the very sparsely distributed unstriated fibers of the bladder. Other decapods act similarly. In a single instance the estuarine crab, *Callinectes hastatus,* immediately on being grasped spurted urine to a distance of about 9 cm. from both nephropores simultaneously. Marchal (1892) noted a distance of 2 cm. from a shrimp and Herrick (1909) "an inch or more" from the American lobster on being held. Herrick ascribed the phenomenon to contractility of the bladder but evidently made no observations to support this supposition.

Whether the sparsely scattered vesicular fibers contribute to the discharge of urine is still unknown. The bladder was subjected to electrical induction shocks of high and low frequency, led through fine Ag–AgCl electrodes, both while distended with urine *in situ* and when isolated and under slight stretch in the longitudinal or in the transverse direction between two points in crayfish-saline. Contraction was never observed even under a magnification of 22.5 ×. The induction shocks were capable of causing cardiac tetanus, contraction of the dorso-anterior and -posterior dilators of the crop-gizzard, of the dorso-posterior longitudinal muscles of the crop-gizzard, and of the intact and isolated intestine, and abduction and adduction of the claw of the cheliped. Electrical stimulation of the bladder frequently produced strong generalised somatic muscular contraction; abrupt flexion of the abdomen and contraction of the homolateral remotor of the second antenna (Fig. 1, *rem.*) were among the main effects. Because the latter muscle is contiguous with the latero-ventral surface of the bladder, its contraction generally falsely suggested contraction of the bladder. Marchal (1892) briefly stated that he was unable to elicit contraction of the bladder of *Maia* by electrical stimulation.

Doubtless the major factor in the expulsion of urine is pressure exerted on the bladder by the blood and crop-gizzard. The nephropores, of animals drained from moisture, were often observed to remain dry for forty minutes or more. The injection of 1 to 1.5 cc. of saline into the haemocoele, between the chelipeds, i.e., in the vicinity of the bladders and crop-gizzard, invariably resulted in an immediate outflow of urine from both nephropores simultaneously. Merely puncturing the integument did not produce effects. If urination was occurring slowly, the injection of 1 to 1.5 cc. of saline resulted in a marked increase in the rate of outflow. It is conceivable that in some instances both bladders may be entirely collapsed; urination then would not be expected even upon the injection of any amount of liquid. Bilateral compression of the integument lateral to the bladders often produced an outflow of urine or an increase in the rate of flow. The large crop-gizzard is partly

wedged between the upper surfaces of the bladders. As direct mechanical pressure on the bladders results in their collapse and in the expulsion of urine, the movements of the crop-gizzard must be a factor in urination.

Innervation

Probably because the kidney and bladder are organs of the second antennal somite, all nerve-fibers to the bladder issue from the tritocerebral lobe of the brain (Fig. 4, *T*). The anterior component of the tegumentary nerves, *te,* sends a branch to the integument beneath the labyrinth; the posterior component sends branches to some of the proximal muscles of the second antenna. About nine fibers issue in the anterior cluster, c_1, which arises from the base of the root of the main antennal nerve-trunks, *a*. Fibers from c_1 innervate the anterior and posterior surface of the bladder. The cluster, c_2, which consists of about five fibers, innervates the posterior surface of the bladder and some of the proximal muscles of the second antenna. Judging from the course of c_2, the sensitive operculum is probably furnished with afferent fibers from c_2 rather than from c_1. The nerve-fibers to the bladder are probably mainly afferent.

Repeated observation could not duplicate, in *Cambarus,* Keim's affirmation (1915; and quoted by Stoll, 1925) that in the European crayfish there extends a nerve-fiber ("nervus glandulae viridis"), bilaterally, from the suboesophageal ganglion to the labyrinth. Keim considered Marchal's description of a renal innervation from the second antennal nerve-bundles as incorrect. Marchal, however, stated that he "could not find a nerve which passed directly to the antennal gland," i.e. without first going to the bladder. Neuwyler (1841) disagreed with the labyrinthic auditory hypothesis of his eminent predecessors, as regards the function of the "green glands," because he could never find a nerve-supply to the glands.[1] Wassiliew (1878), in one of the first papers on the histology of the decapod kidney, stated that no nerves could be seen to enter the kidney. The present writer's observations are in accord with Wassiliew in this respect. The absence of a nerve-supply at the kidney proper indicates that secretion by this organ is not influenced by the

[1] To Ernst Haeckel (1857) credit is due for first suggesting that the green glands are renal organs. Haeckel demonstrated the communication of the bladders with the exterior and with the glands by introducing metallic mercury into the bladders. He pointed out that the existence of an external orifice indicates that the liquid in the bladder is a secretion which is eliminated. This observation, together with Neuwyler's discovery of the tubule and Gorup-Besanez and Will's remark that guanine occurs in the green glands, led Haeckel to term these glands urinary organs. Gorup-Besanez and Will, however, did not state the concentrations of guanine in urine and blood.

nervous system. This is supported by Maluf, Clarke, and Thompson (1939), who were the first to show that, per unit volume of glomerular filtrate, the rate of secretion of various substances is identical in the denervated and normal mammalian kidney.

The Absence of a Valve between the Nephric Tubule and the Bladder

The epithelial cells of the bladder, except those of the most proximal portion, are never columnar. They may be highly vacuolated (Fig. 9, *A* and *B*) or plain (Fig. 9, *C*) in the same bladder. The physiological evidence indicates that the epithelium of the main body of the bladder is non-secretory (Maluf, 1941*b*).

Even though the columnar secretory epithelium of the distal half of the nephric tubule (Fig. 7, *dt* and Maluf, 1939) merges imperceptibly into the epithelium of the main body of the bladder (Maluf, 1939), the tubule as an organ ends abruptly (Fig. 7), since the epithelium of the bladder is not anastomosed and acutely involuted as is that of the tubule (Fig. 8). The distal orifice of the tubule can be readily observed *in situ* (Fig. 8) through the dorsal surface of the translucent distended bladder. There is no valve between the tubule and bladder (Fig. 8) and no evident constriction of the proximal end of the bladder. Sections show no valve or sphincter at the distal orifice of the tubule.

The bladder of a 14-gram animal normally can distend to a diameter of at least 8 mm. The hydrostatic pressure within the bladder must then be somewhat greater than that of a column of water 8 mm. high because the bladder is elastic (see above). This pressure is doubtless too low to interfere with the outward secretion of water for which evidence is presented in an accompanying paper (1941*a*).

The Absence of a Valve and Sphincter between the Coelomosac and Labyrinth

The entire series of sagittal and horizontal serial sections of two kidneys, fixed in formol-sublimate and stained with haematoxylin and methylene blue, was studied. No valve or fibers could be found. A sagittal section at the communication of the lumina of labyrinth and coelomosac is shown in Fig. 10.

The Epithelium of the Coelomosac

The epithelium of the coelomosac, like the rest of the nephron, is single-layered. The appearance of more than one layer throughout a

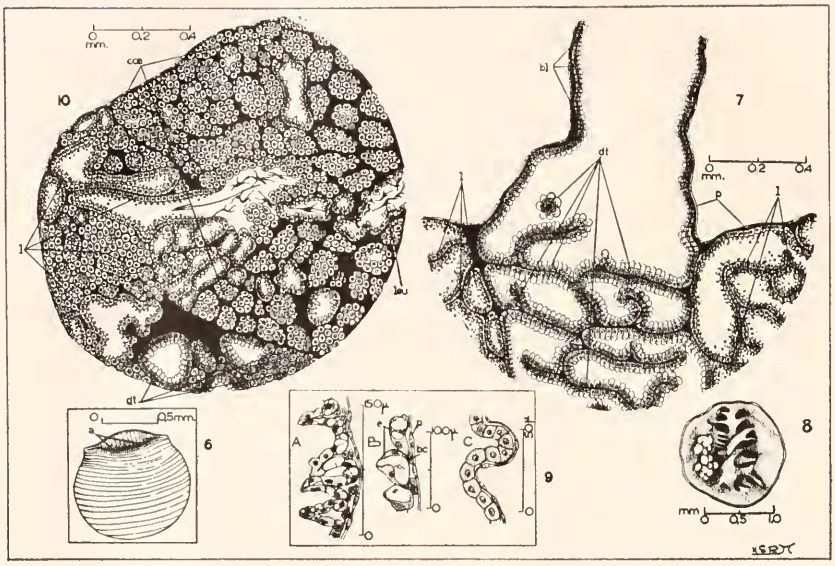

PLATE II

Fig. 6. Dorsal aspect of left nephropore, *a*, and membranous operculum. Note diagonal position of the nephropore. Animal, 22 grams.

Fig. 7. Sagittal section through the kidney showing communication of the distal extremity of the tubule, *dt*, with the bladder, *bl*. *l*, labyrinth; *p*, peritoneum. Solid black areas indicate blood-sinuses and blood-vessels. Animal, 35 grams.

Fig. 8. Dorsal aspect of the distal orifice of the tubule at its communication with the bladder.

Fig. 9. Sections of the main body of a single bladder. *bc*, blood-cells; *e*, epithelium of bladder; *p*, peritoneum. Animal, 35 grams.

Fig. 10. Sagittal section through the kidney showing communication of the coelomosac, *coe*, with the labyrinth, *l*. *dt*, distal portion of the tubule; *leu*, leucocyte. Solid black areas indicate blood-sinuses and blood-vessels. Animal, 35 grams.

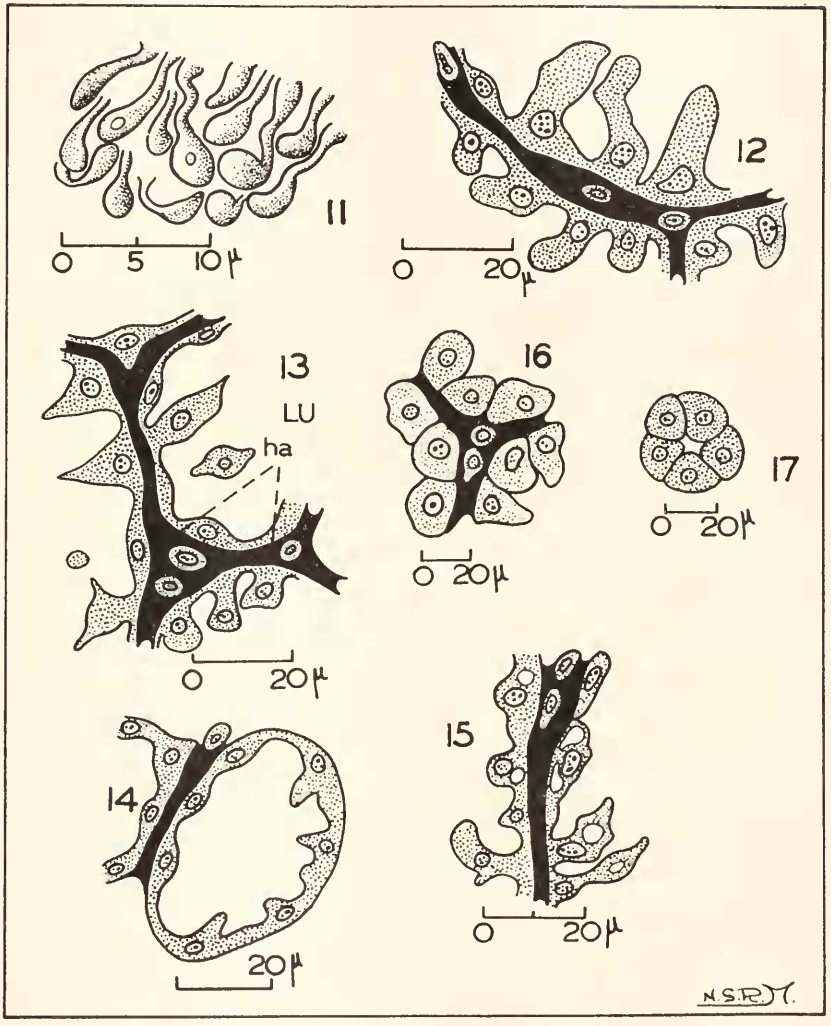

PLATE III

FIG. 11. "Living" portion of the coelomosac teased from the kidney in crayfish-saline and suspended in a hanging drop of crayfish-saline. The apical bulbous protuberances of the cells are shown in relief.

FIGS. 12 to 17. Epithelium of the coelomosac from medium-sized individuals. *ha*, haemocoele; *LU*, lumen of coelomosac. See text.

large part of the coelomosac, labyrinth, and tubule (Fig. 10) is doubtless due to tangential sectioning.

The nephron of the crayfish has no tenuous syncytium, such as the glomerular capsule of the vertebrate nephron. The epithelium of the coelomosac, the most promixal organelle of the nephron, approaches nearer to being a narrow syncytium than any other part of the nephron (Figs. 12–15). In several of the individuals examined, however, the cells were large and rounded throughout the coelomosac (Figs. 16 and 17; and Maluf, 1939).

The epithelium of the coelomosac varies considerably not only from one individual to another but often, too, in a single animal (Figs. 11–17). The cells may be either compact, rounded, and sometimes vacuolated, as in Figs. 16 and 17, or more or less squamous with large protuberances directed into the lumen (Figs. 12–15). The cells at the periphery may be rounded while those toward the center are protuberant; the reverse has never been found. Frequently, either the protuberant or the rounded cells occur exclusively. Both coelomosacs of an individual are always identical.

The histological methods have been described in the previous paper (Maluf, 1939). The protuberant type of cell is evidently not an artefact because it has been observed in teased-out "living" fragments in a hanging drop of crayfish-saline (Fig. 11). The composition of the saline is stated elsewhere (1941*a*).

Grabowska (1930) claimed that the secretion of the coelomosac consists of a discharge of cells in their entirety, i.e. "holocrine" secretion. If the cells are discharged as a whole, one would expect them to be substituted by mitosis. In not one instance, out of numerous coelomosacs examined, has the writer been able to find a mitotic figure. The evidence for a discharge of globules from the apical region of these cells is dubious because where rounded bodies have been seen "free," in the lumen of the coelomosac in sectioned preparations, these may have been merely sections of the bulbous tipped protuberances. In contrast to the rest of the nephron, the main lumen of the coelomosac generally contains numerous leucocytes (Fig. 10, *leu*).

Upon teasing the kidneys of a crayfish on one occasion, the coelomosacs were found packed with hard yellowish-brown irregular concretions the size of which showed that they could not have been intracellular. The largest stone was about 0.2 mm. in length. The material was insoluble in cold and hot water and in absolute ethyl alcohol. The alcohol decomposed the surrounding yellowish-brown organic material and the white stones readily fell apart, upon contact, into minute needle-like crystals which did not dissolve. The stones readily dissolved in

dilute HCl with energetic release of a colorless gas and were slowly soluble in 10 per cent NH_4Cl. There is very little doubt, therefore, that these concretions were $CaCO_3$. The individual had a highly melanized abdominal venter and, hence, must have possessed well-developed calcareous gastroliths. Twenty-one crayfish with gastroliths were examined and only one exhibited a similar condition. This was a single fairly large concretion in the coelomosac of only one kidney; other parts of the kidney did not contain any stones. About thirty animals with a light abdominal venter and without gastroliths were examined and in no instance was any concretion found in the kidneys.

The concentration of calcium in the blood of the crayfish and crabs remains fairly constant even immediately after molting (Paul and Sharpe, 1916; Damboviceanu, 1930), i.e. even when there is occurring, by way of the blood, a rapid transfer of calcium from the hepatopancreas and/or gut to the integument. Oesterlen (1840) has suggested that the formation of gastroliths may be a way of preventing a rise in the concentration of calcium in the blood. The above instances of renal calculi may be exceptions that prove the possible rule that one of the functions of the coelomosac is the secretion of calcium from the blood.

Weismann (1874) suggested that a blood-ultrafiltrate is formed through the coelomosac of the crustacean nephron just as Ludwig (1844) had presumed to occur through the glomerular capsule of the vertebrate nephron. Grobben pointed out that the relatively simple coelomosac of various amphipod crustaceans is attached to the integument by strands; this fact supports Weismann's belief inasmuch as effective resistance to blood-pressure would thereby be offered by the coelomosac, which would otherwise float in the haemocoele. Grobben also suggested that the location of the coelomosac between the antennal muscles in phyllopod Crustacea favors filtration. He nevertheless pointed out that, in copepod Crustacea, the coelomosac lies freely at the entrance to the homolateral second antenna and that these animals have no heart; he also drew attention to the fact that the phyllopod Crustacea have no heart and that it is therefore questionable whether, in such instances, filtration can occur and he ascribed the formation of urine in copepods and early-instar phyllopods to secretion by the tubule—a conception which had just begun to gain favor due to Heidenhain's (1874) experiments with the mammalian kidney.

Certain teleological evidence contra-indicates filtration through the coelomosac. Marshall and Smith (1930) and Marshall (1934) pointed out that when fishes migrated from freshwater, where they evidently arose, into the sea they had to conserve water. Some succeeded in losing their glomeruli while others are still doing so. The crayfish, how-

ever, has probably descended from a marine ancestor and is capable of compensating for water which diffuses inwardly through the gills (Maluf, 1937) by manufacturing a hypotonic urine through the agency of its nephric tubule. The crayfish nephron has a coelomosac but so does that of the lobster—a strictly marine relative. Because the osmotic pressure of the blood of the lobster is slightly hypertonic to that of the surrounding sea water (Cole, 1940), the lobster, unlike the crayfish, does not absorb water by diffusion from the exterior and hence does not have to maintain a steady state by an outward secretion of water. The lobster has either lost its nephric tubule or has never owned one. If the coelomosac were a filtration-organelle one would expect it to show some signs of regression in the lobster; but the coelomosac of this crustacean exhibits no evidence of being on the decline. Physiological evidence (Maluf, 1941*b*) indicates that the nephron of the crayfish is paramountly if not entirely a secretory organ.

Summary

1. The internal anatomical features surrounding the urinary outlet of the crayfish are described in detail for the first time.

2. Urine is retained in the bladders evidently by the ureteral syncytium, which is here described for the first time. There is no other way, conceivable to the writer, by which urine can be retained. Fibers do not insert on the operculum of the nephropore.

3. Urine is discharged by a localized rise in the haemocoelic pressure and can be expelled by direct action of the crop-gizzard on the bladders. Adequate electrical stimulation cannot cause contraction of the bladder but often evokes generalized motor activity.

4. Occasional abrupt spurts of urine, which were almost simultaneous from both nephropores, extended to the distance of a foot or more.

5. Destruction of the opercula before urinary collection has no rationale.

6. The bladder is innervated by fibers from the tritocerebral lobe of the brain. These fibers are doubtless mainly if not entirely afferent. The kidney is not innervated.

7. There is no valve between the nephric tubule and the bladder.

8. There is no valve or sphincter between the coelomosac and the labyrinth.

9. The epithelium of the coelomosac, the most proximal portion of the nephron, has been studied in detail. "Holocrine" secretion evidently does not occur because no mitotic figures could be found. The histological, chemical, and phylogenetical data contra-indicate filtration through the coelomosac.

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